APPLIED MICROSCOPY

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The Aberdeen Proving Ground in general and the Ballistic Research Laboratories in particular are interested in the manner by which absorbed energy is utilized by various systems. The technique describes the possibility of an evaluation of the traumatic effect brought about by the absorption of energy at the cellular level.

This paper (presented, in brief, to the Eighth Conference on the Design of Experiments in Army Research, Development and Testing, The Walter Reed Army Institute of Research, Washington, D. C., October 1962) has to do with the ultraviolet microscopy of biological tissues.

There is much controversy having to do with the structure and function of biological cellular configurations. This controversy will continue to exist until methods are employed for the analysis and evaluation of experimental results which do not (by preparation and/or examination) change the materials during the evaluation procedure.

A paper describing a photomicrographic technique was published by Köhler (1) in 1904. The optical system was quartz instead of the usual glass. The purpose of this was to take advantage of the increase in resolution which is realized when ultraviolet light is used as the source of illumination instead of the visible region of the spectrum.

The light source employed by Köhler was a condensed electrical spark between metallic electrodes. This is unsatisfactory for the illumination of a microscope. The electrodes burn away non-uniformally which means that it is difficult to keep an image of the source centered on the microscope condenser. Also the noise of the spark is objectionable. In spite of these and other difficulties, some work has been published (2).

The early workers were attracted to this field because the technique offered the possibility of considerable ultimate magnification (since the resolution was about twice that obtained with visible light) although the initial magnification might be low.

In other words, the increase in resolution made possible the visualization of microscopic structure which was not evident until it was revealed by the enlarging camera.

It is to be pointed out that the obvious lack of light intensity was due to the inefficiency of the monochromator system used to isolate the wave length of light (2750A) for which the objectives were corrected.

It was also realized that tissue components such as proteins, nucleic acids and nucleoproteins have specific absorption bands in this region of the spectrum so that it should be possible to obtain micrographs of fresh and unstained material.

Thus, in a sense of the word, it can be said that the chemical constituents of the tissue can act as their own specific light absorbing medium -- the stain.

It is well understood that the fixation process (necessary for the manipulation of tissue cutting) may contribute to the micrographical picture. We do not always know what contribution this represents to our over-all picture. By means of frozen sections we can minimize this uncertainty. We will also need the frozen section-type of section for our analytical procedures.

The inherent objection to previous procedures was that it was not possible to obtain sufficient light intensity of the proper wave length (in the plane of the photographic plate) so that a field and focus could be localized and imaged with the wave length of light which is specifically absorbed by the material under examination.

In some cases, a fluorescent plate was placed directly over the microscope eyepiece. Thus another adjustment had to be made in order to bring the image in focus in the same plane of the photographic plate. In other words, the focus must be in the plane of the photographic plate.

In 1943 a short paper on the subject of ultraviolet microscopy was published by Lavin[3]. A procedure was described whereby an image of the material on the stage of a microscope could be visualized by a fluorescent screen which was temporarily placed in the position usually occupied by a photographic plate. That is, in the plate holder.

The 2537 Angstrom mercury line was the light source (loc. cit.). It has been previously pointed out that this wave length of light is in the region of absorption of those chemical compounds which are to be associated with tissue structure.

The photographs which are now shown will serve to illustrate this technique and some of the results obtained by application of this procedure. The original plates were taken at a magnification of about 200 diameters.

DESCRIPTION OF PLATES

1. Ultraviolet photomicrographic apparatus described by Köhler. Note the spark source, the monochromator and the eyepiece focusing attachment.

2. Apparatus used in the present work. Light source, liquid filter, willemite focusing screen.

3. Light source -- quartz resonance radiation lamp.

4. Spectrum of light source, with and without filter.

5. Fresh Hamster muscle -- teased out (not cut), fresh, unstained.

6. Muscular dystrophy -- cross section.

7. Muscular dystrophy -- longitudinal.

8. Fresh smear of cells from a chicken egg.

9. Liver, normal -- fixed, unstained.

10. Liver, infectious hepatitis, fixed, unstained.

11. Cross section of a plant root (Sorrell), unstained.

12. Cross section, skin (mal del pinta), unstained.

13. Salivary gland of a mosquito, fresh -- unfixed, unstained.

14. Enlargement of a portion of the salivary gland shown above.

15. Kidney, cross section -- unstained.

16. Red cells, smear, monkey, dried.

17. Red cells, smear, chicken, dried, showing nuclei.

18. Arbacia eggs, showing the "relayering" on rupture.

19. Arbacia eggs, showing the results of photography in the visible, ultraviolet and infrared regions of the spectrum.

20. Muscle photographed using the 2537 A^{o} mercury line, unstained, visible -- stained, desicated.

21. Absorption spectrum curve of 1, 2 -- Benzanthracene.

22. Absorption spectrum of the same substance using the continuous light from a hydrogen discharge tube as the light source.

23. Apparatus for photomicroscopy -- three light sources, infrared, visible, ultraviolet.

THE USE OF CONTINUUM AS A LIGHT SOURCE FOR ABSORPTION SPECTRA. As has been pointed out those compounds such as proteins nucleic acids, nucleoproteins etc. have broad absorption bands when measured by spectrophotometers.

It has also been shown by Lavin and Northrop[4] and others that a considerable amount of band structure can be demonstrated (at room temperature) by the use of a spectrograph of comparative low dispersion. It was also indicated that these bands can be interpreted in terms of the component parts of the molecule by Lavin, Loring and Stanley[5]. This technique has also been applied to complicated mixtures (body fluids) by Dobriner, Lavin, and Rhoads[6 & 7].

Photographs illustrating the above are shown in Plate 22. It is thought that efforts to apply this technique in obtaining the absorption spectra of materials on the stage of a microscope might be worthwhile. This could be a clue to the chemical composition of the various sections of material under examination.

SUMMARY. Photomicrographs of fresh and of unstained tissues obtained with the 2537 A° mercury line as the light source are shown and some of the implications of this technique are discussed.

The possibility of a microspectrographic application to the problem is considered.

ACKNOWLEDGEMENT. The photomicrographs of tissue which were made while the author was at the Rockefeller Institute for Medical Research are used with the kind permission of Dr. Detlev W. Bronk.

REFERENCES TO PREVIOUS WORK

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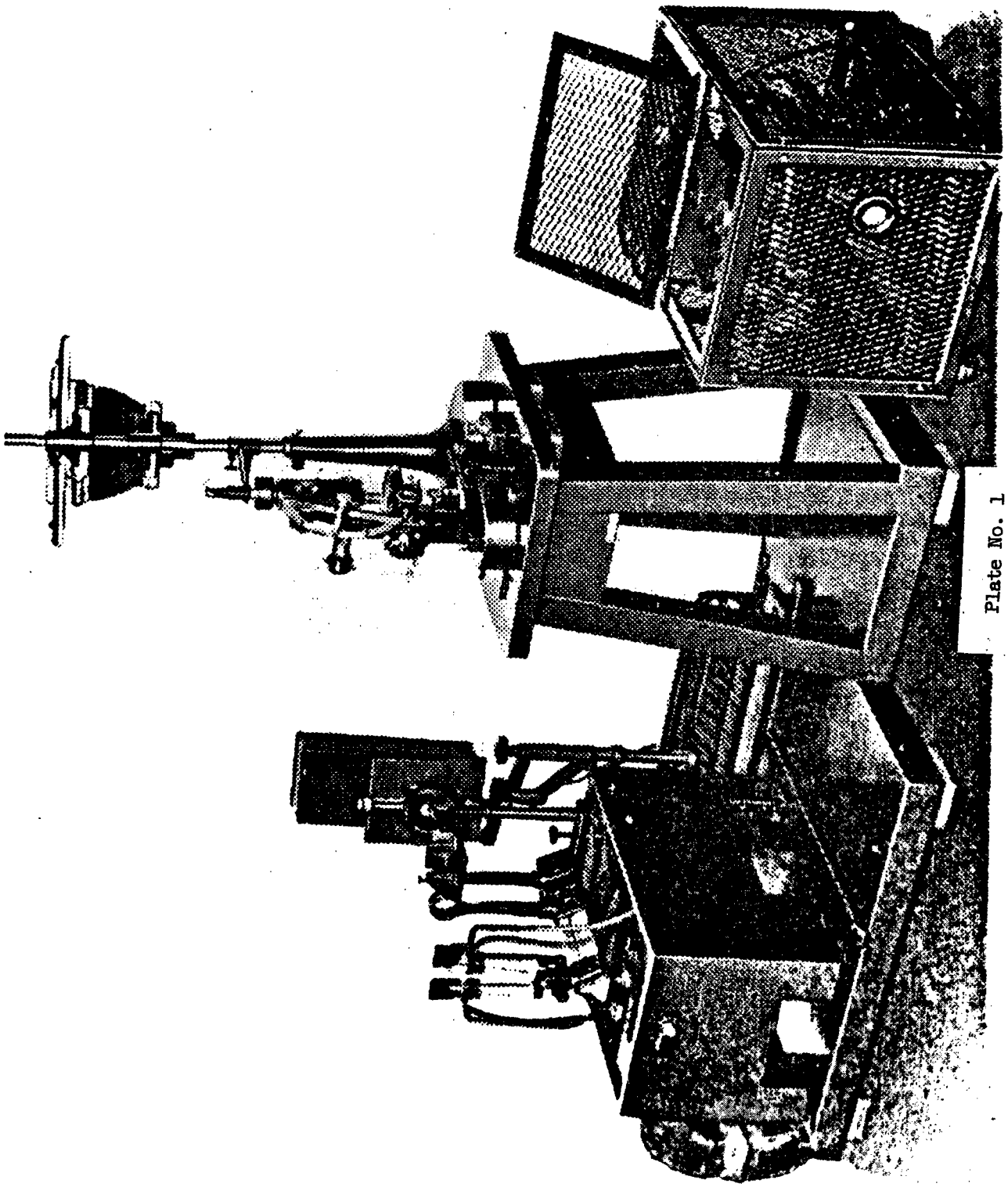
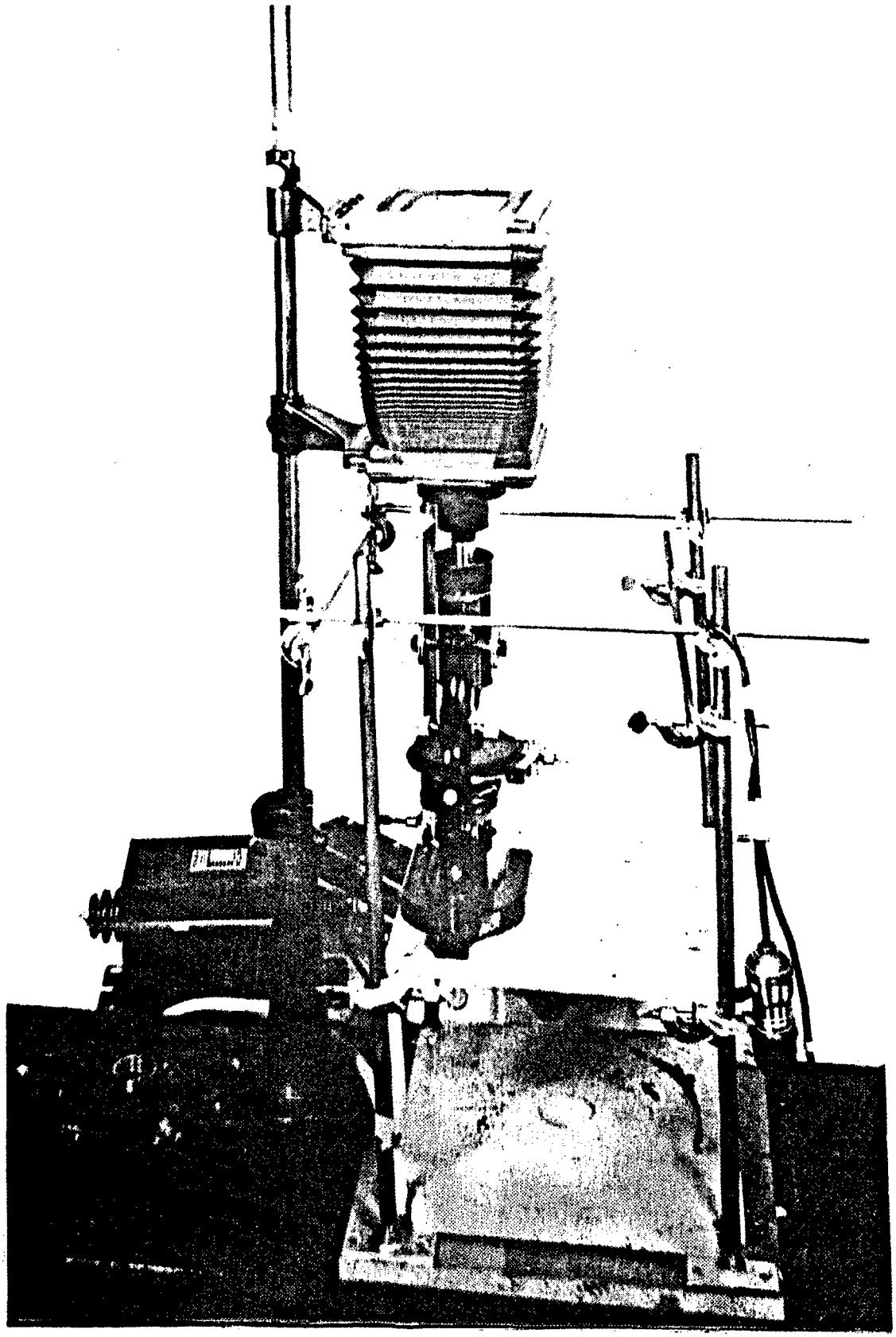


Plate No. 1



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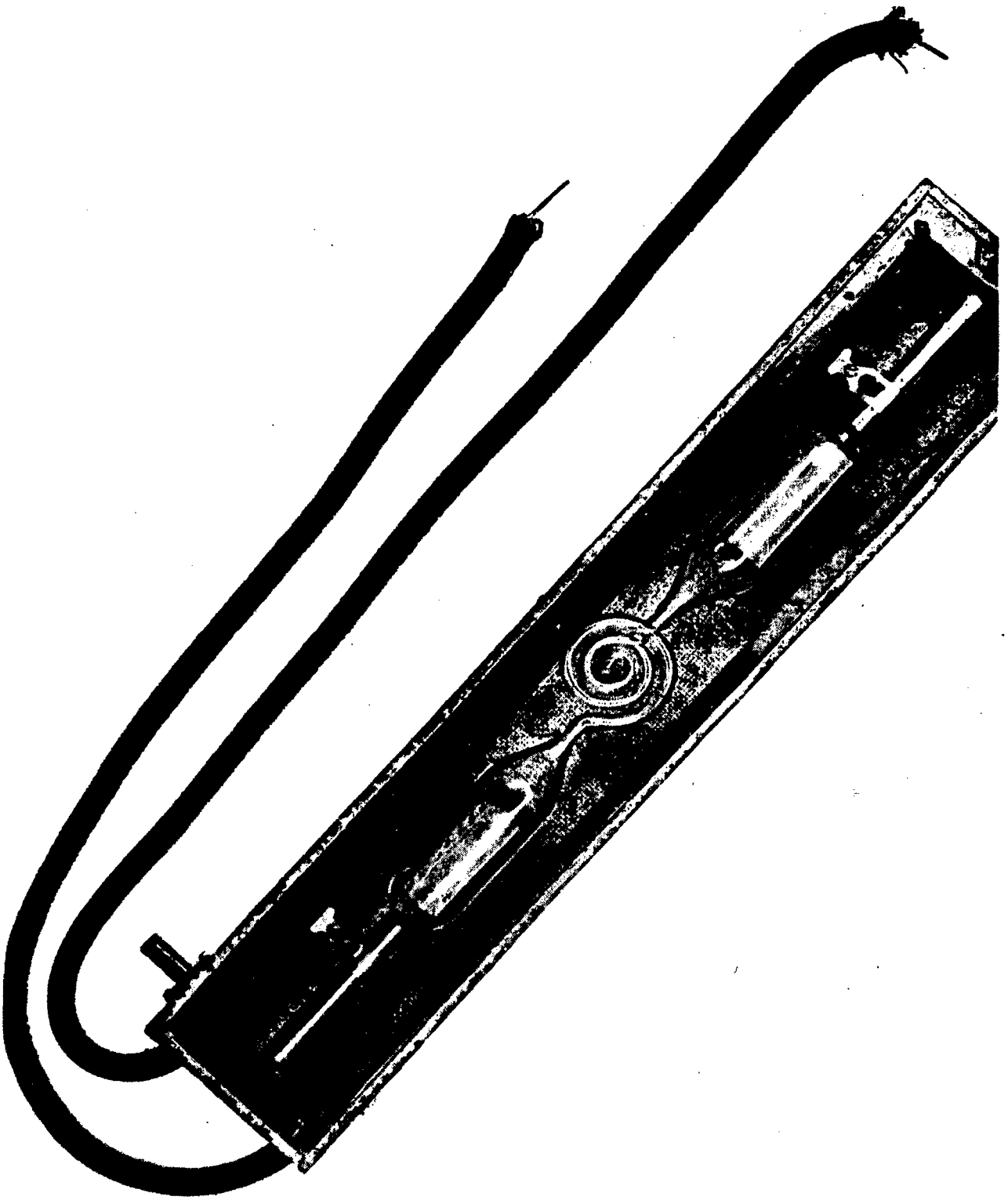

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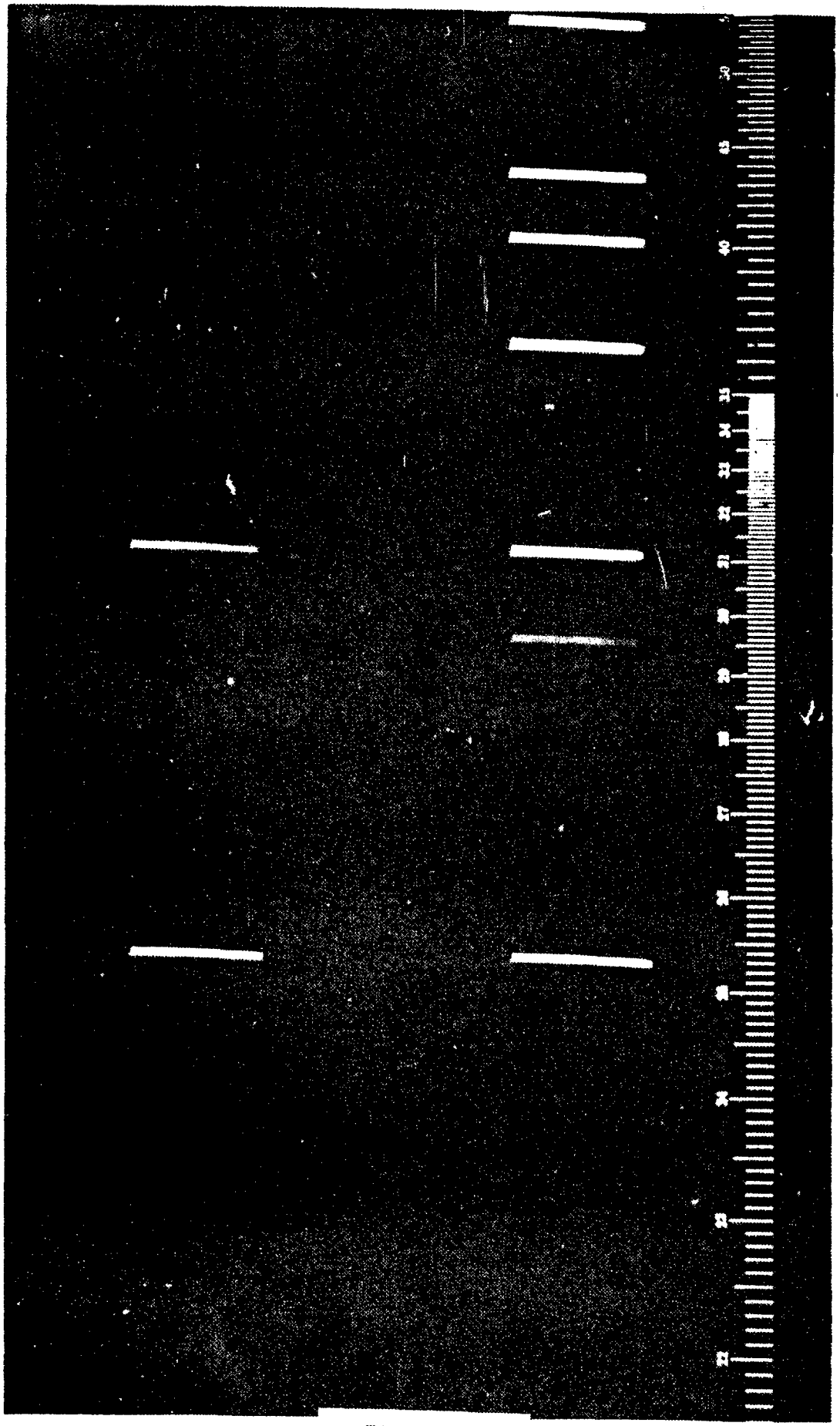

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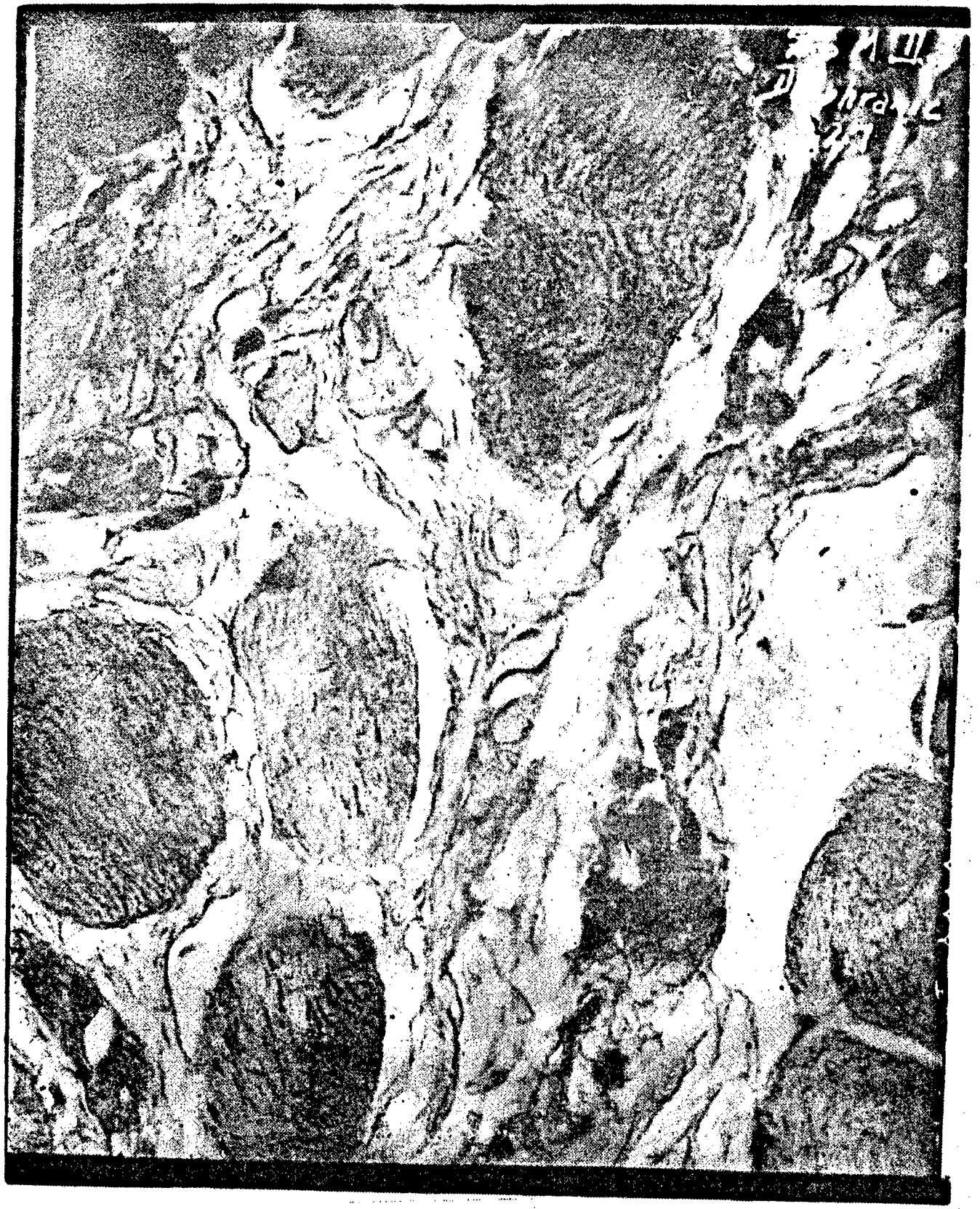

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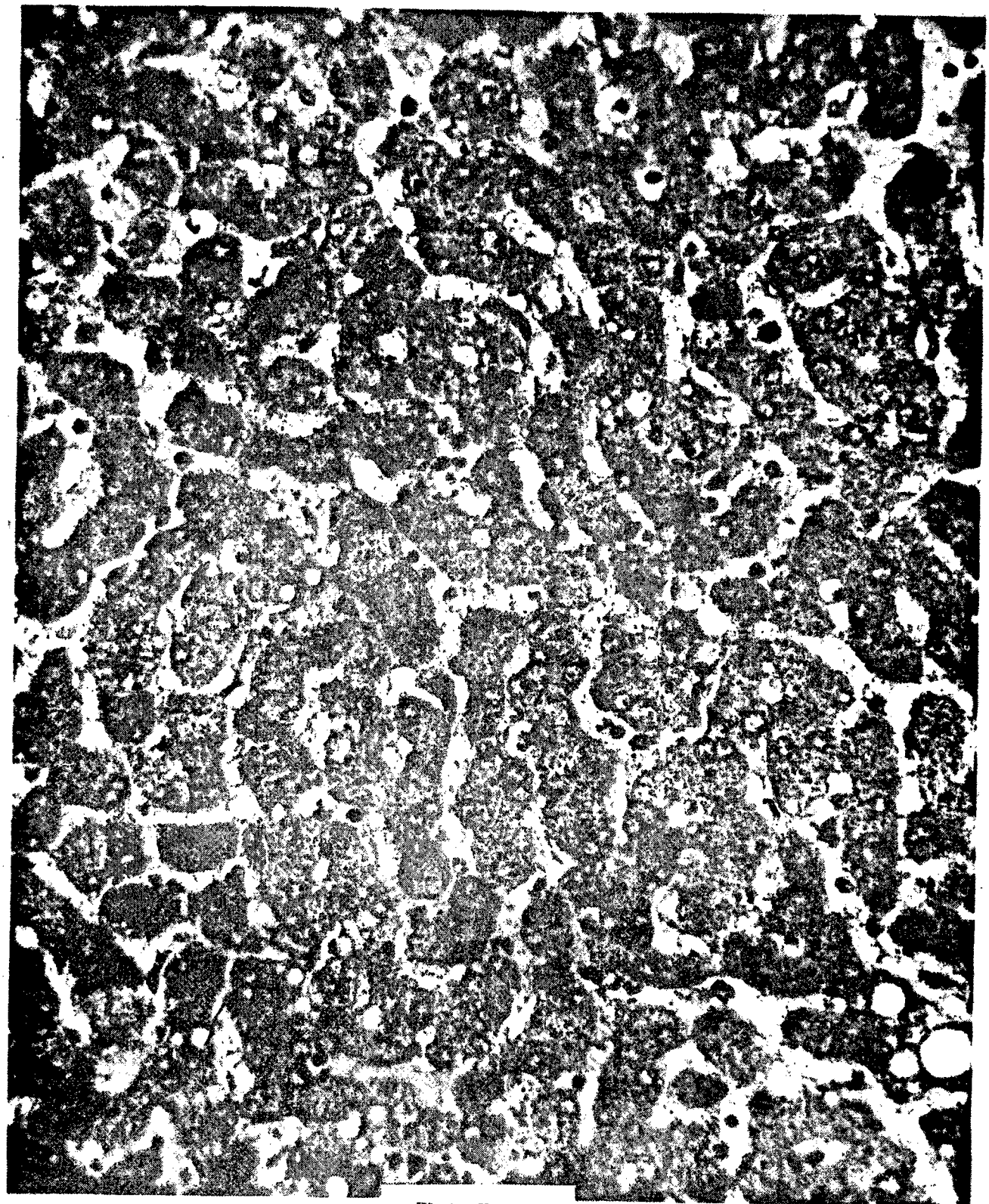
Plate No. 7

Plate No. 8



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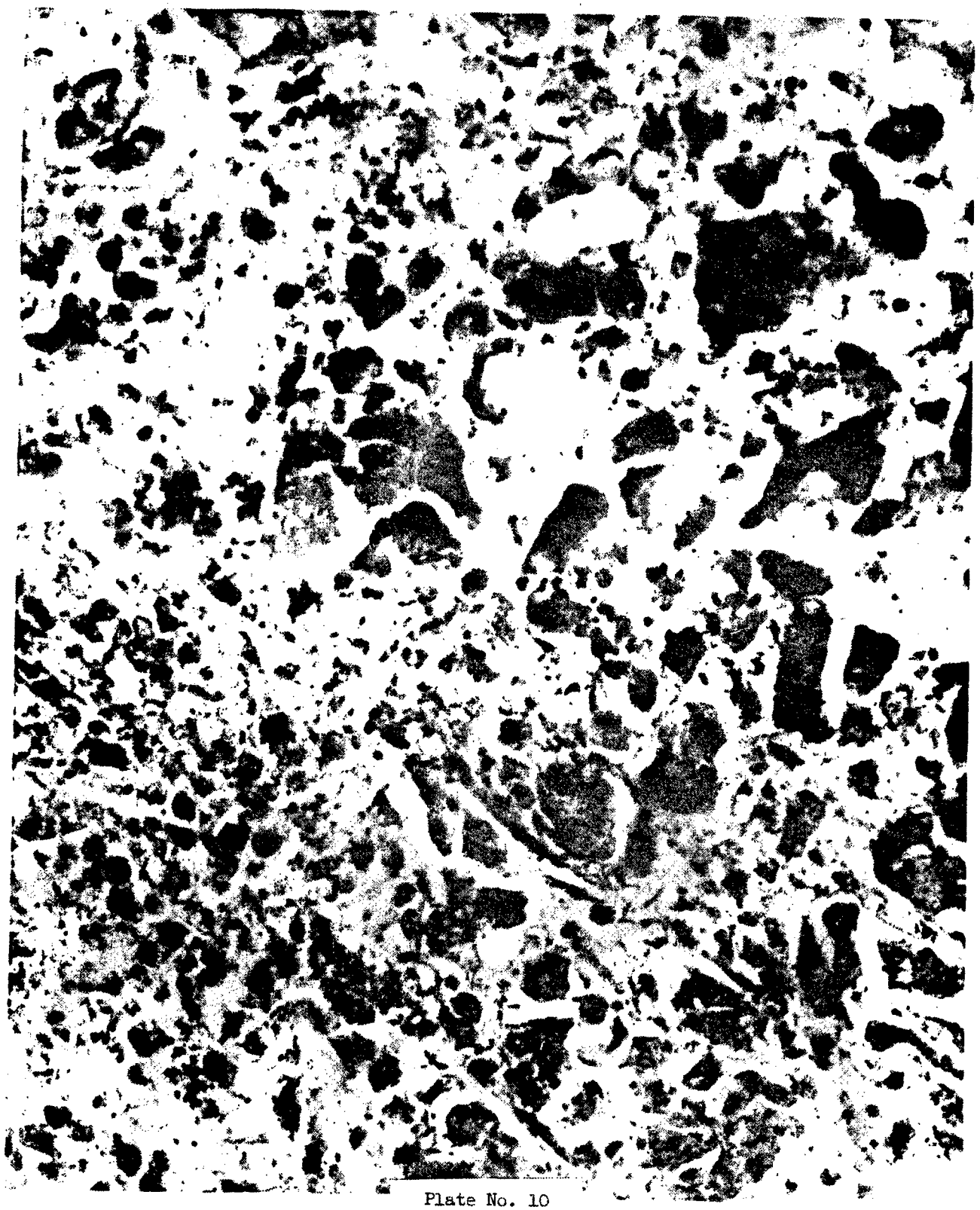

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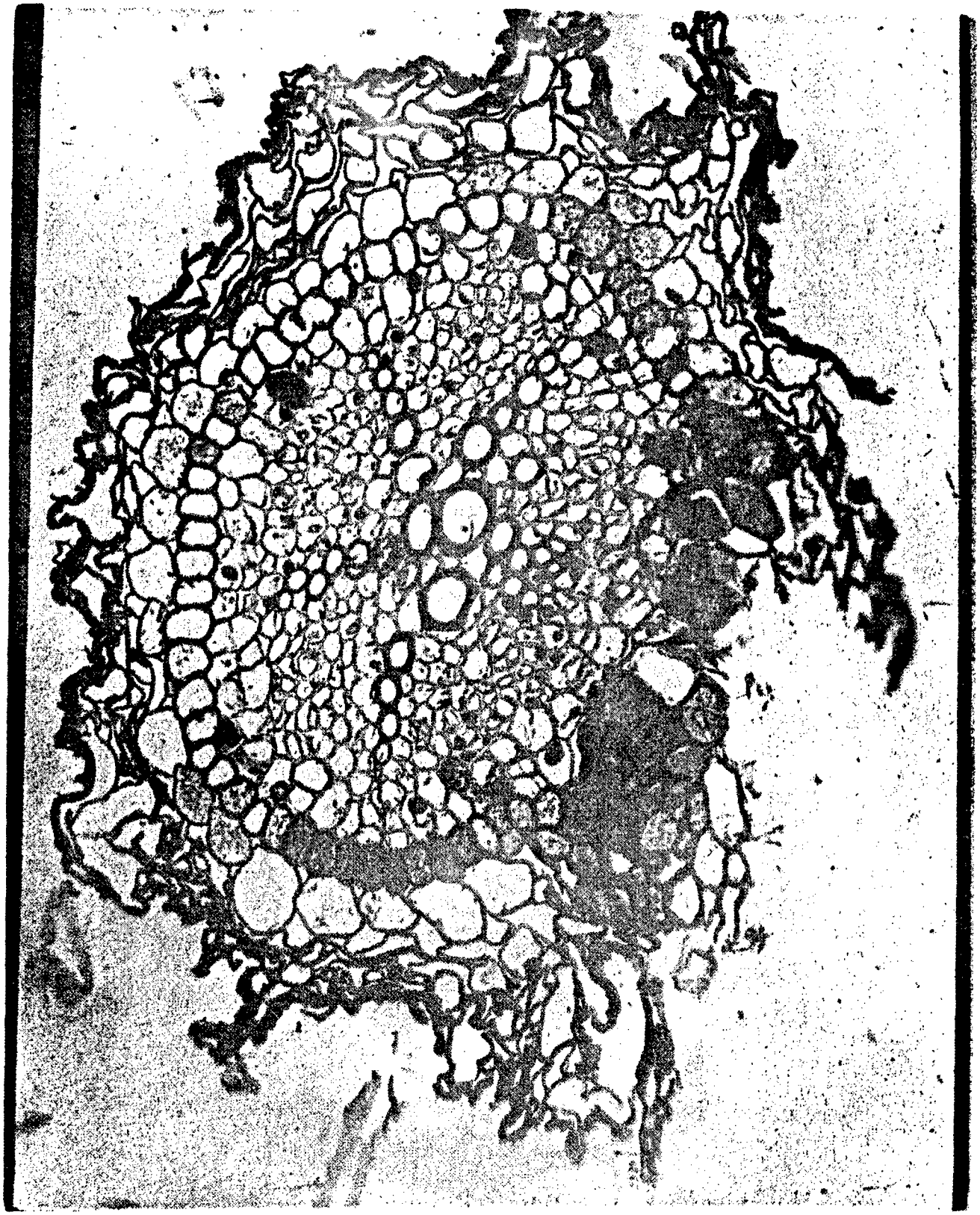

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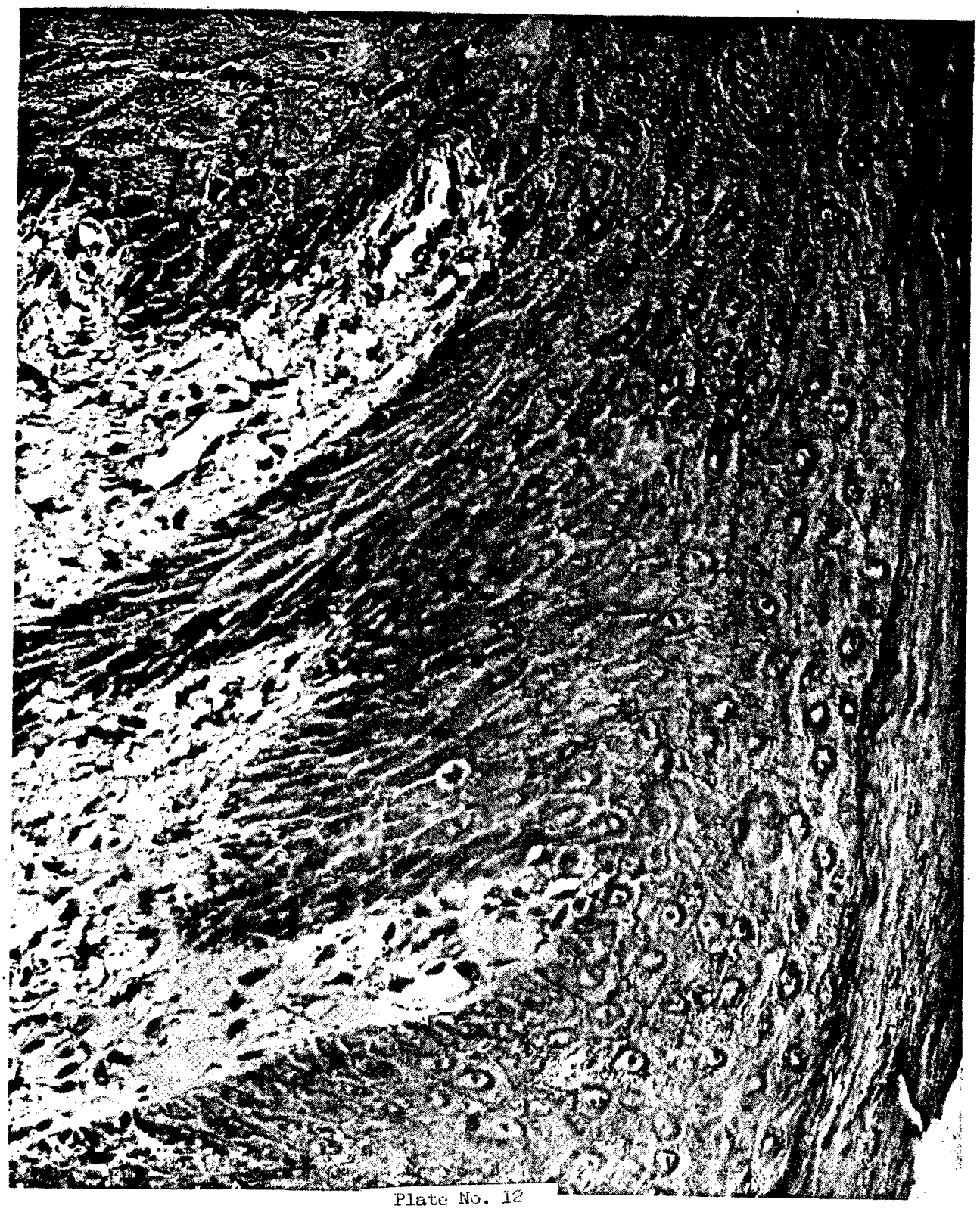

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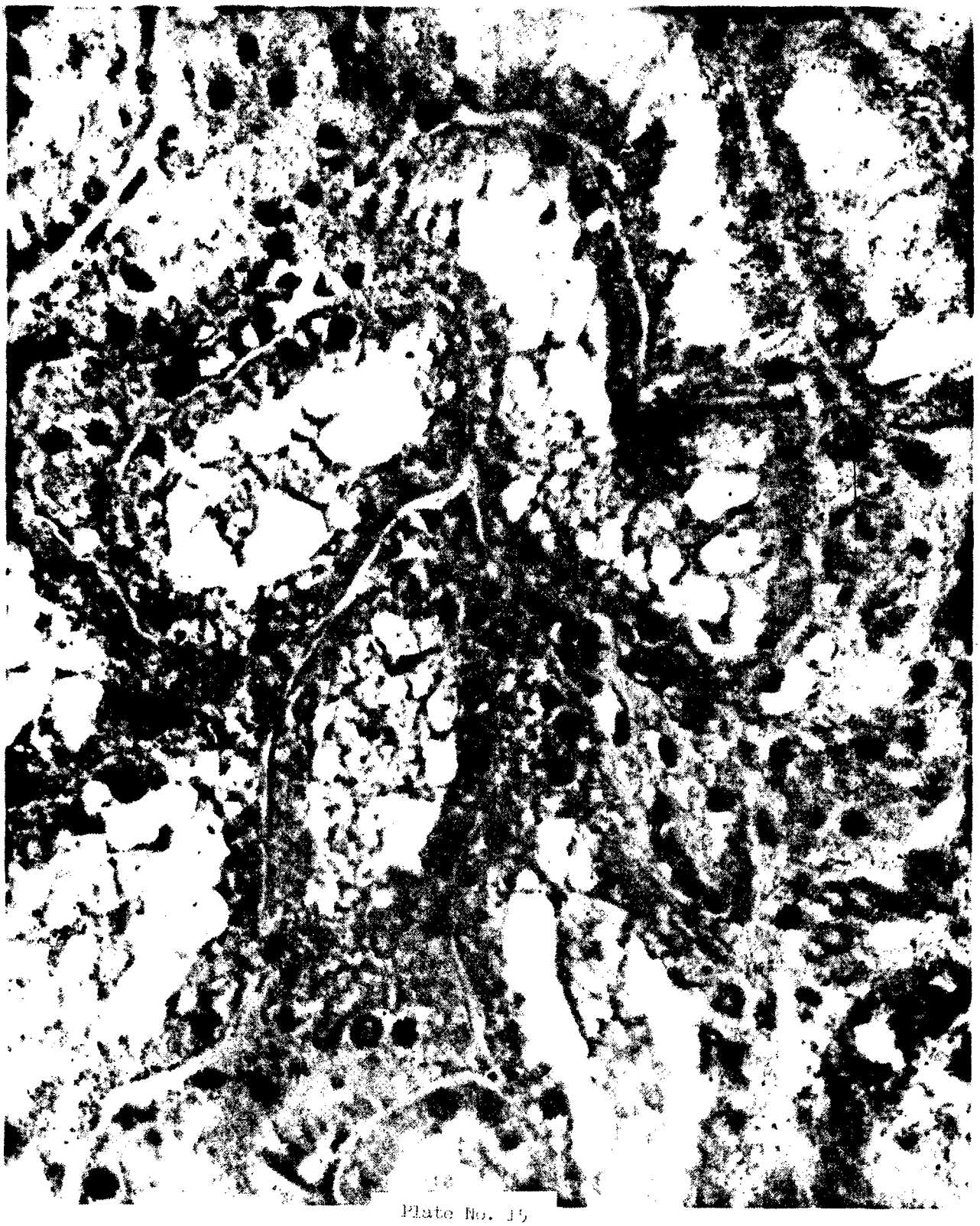
Plate No. 13

Plate No. 14



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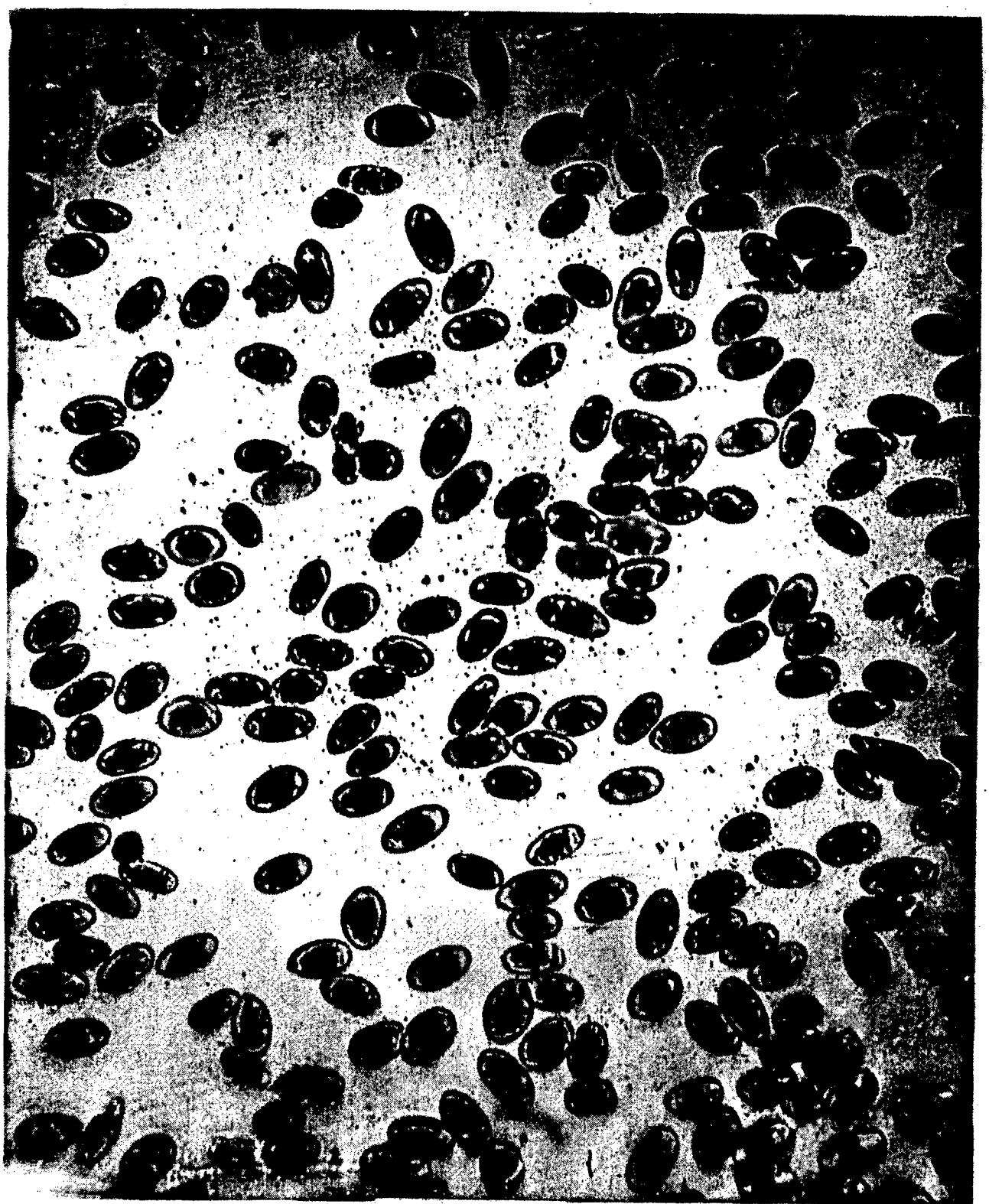

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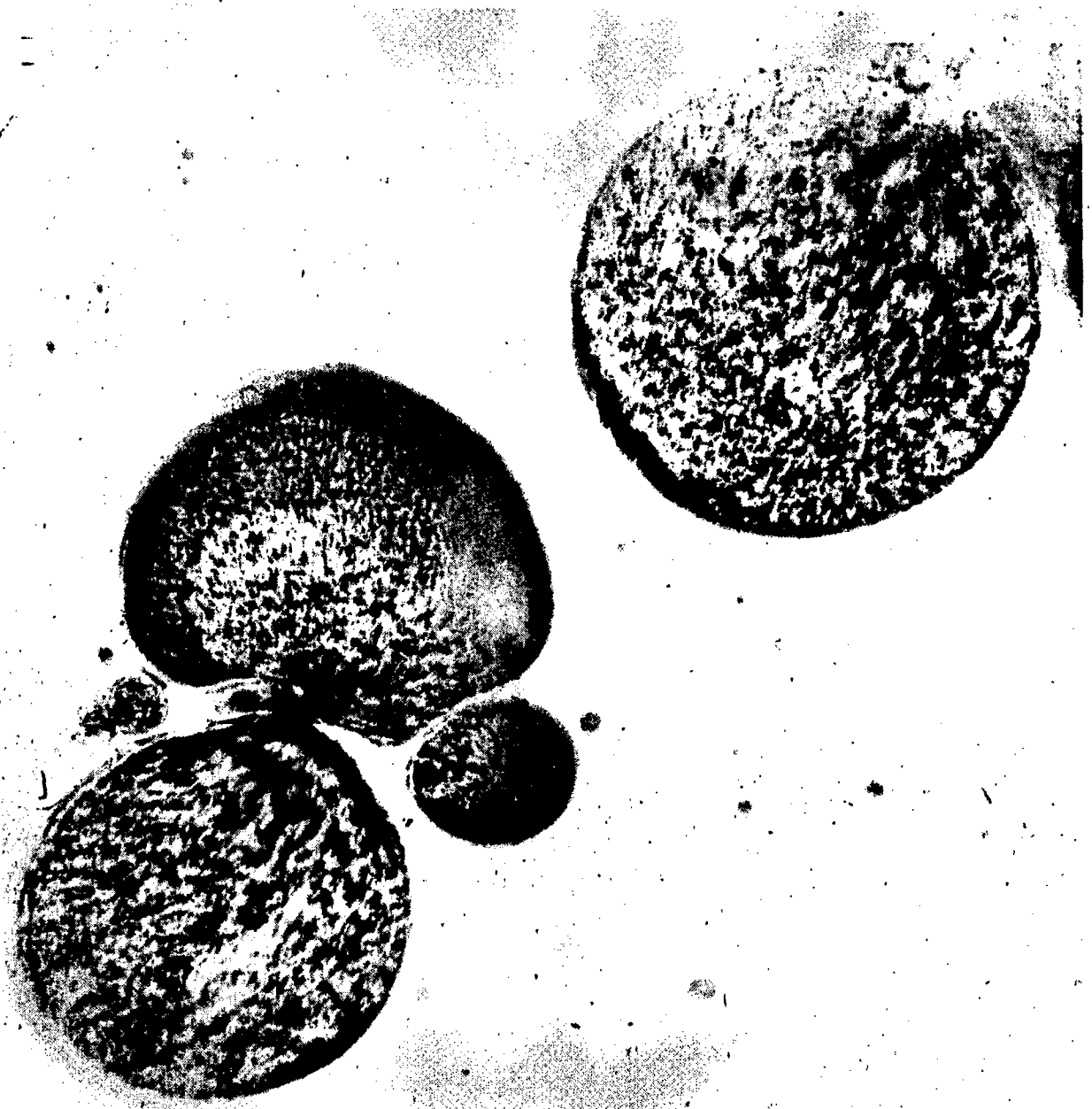

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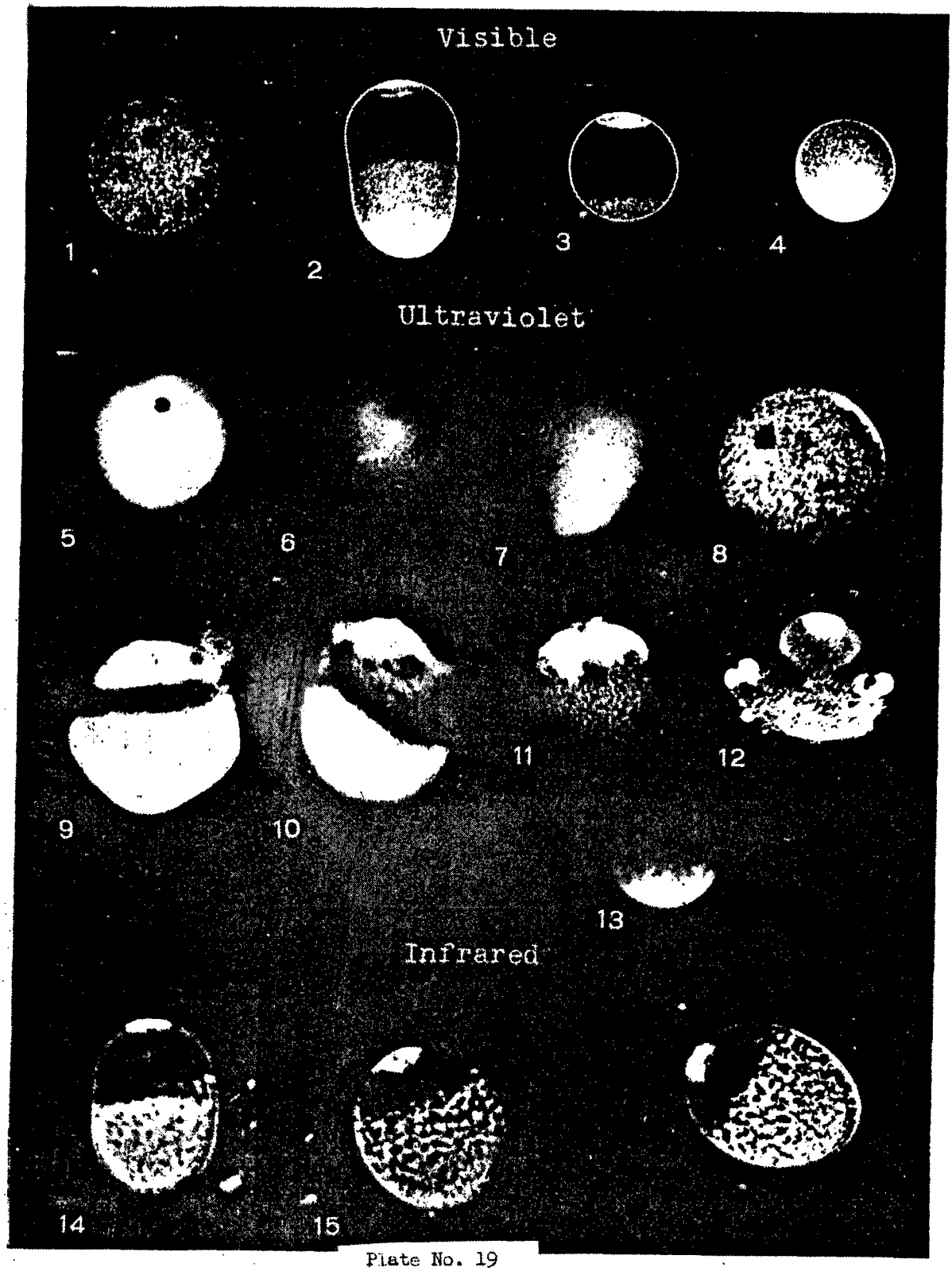

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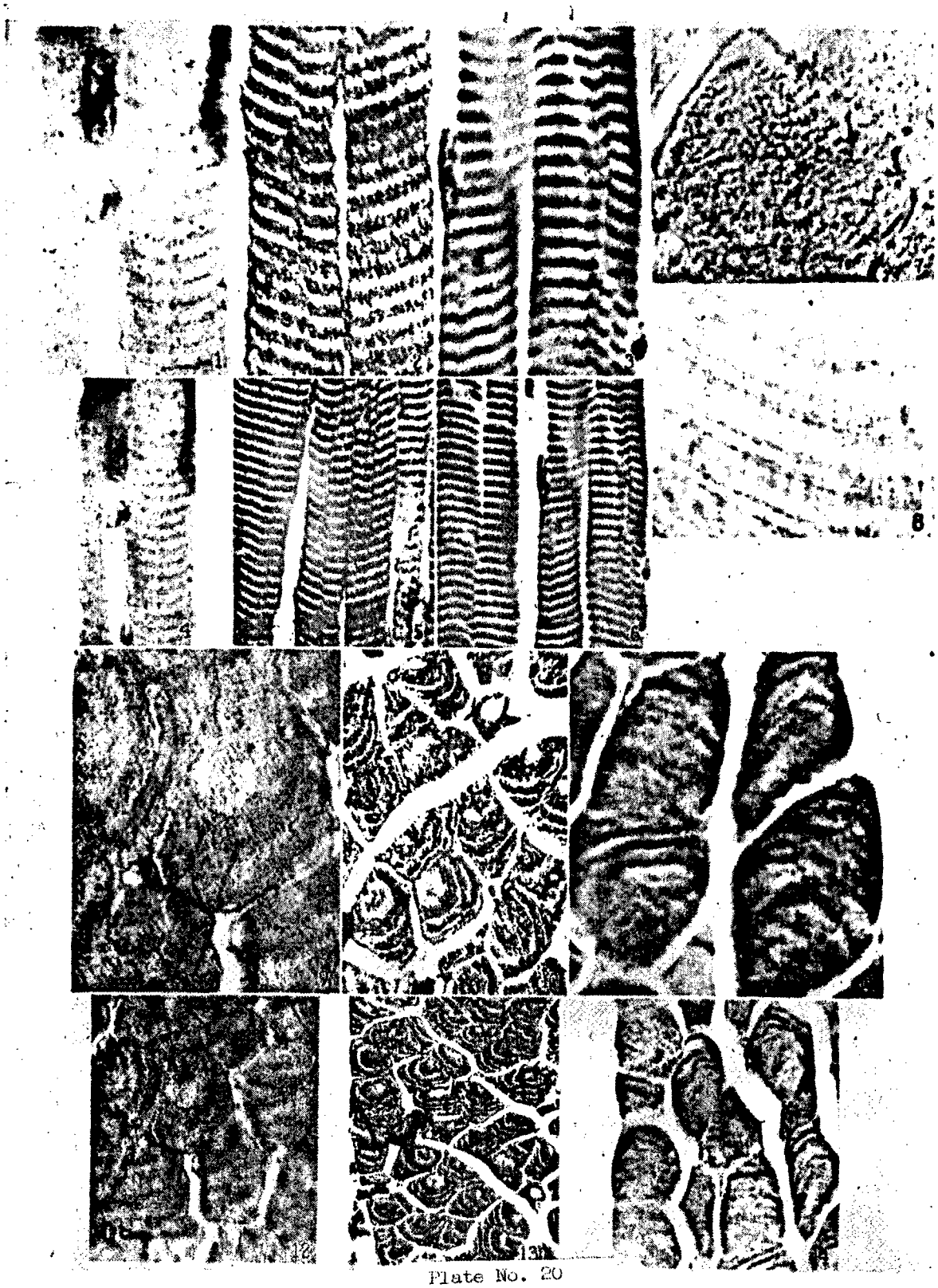

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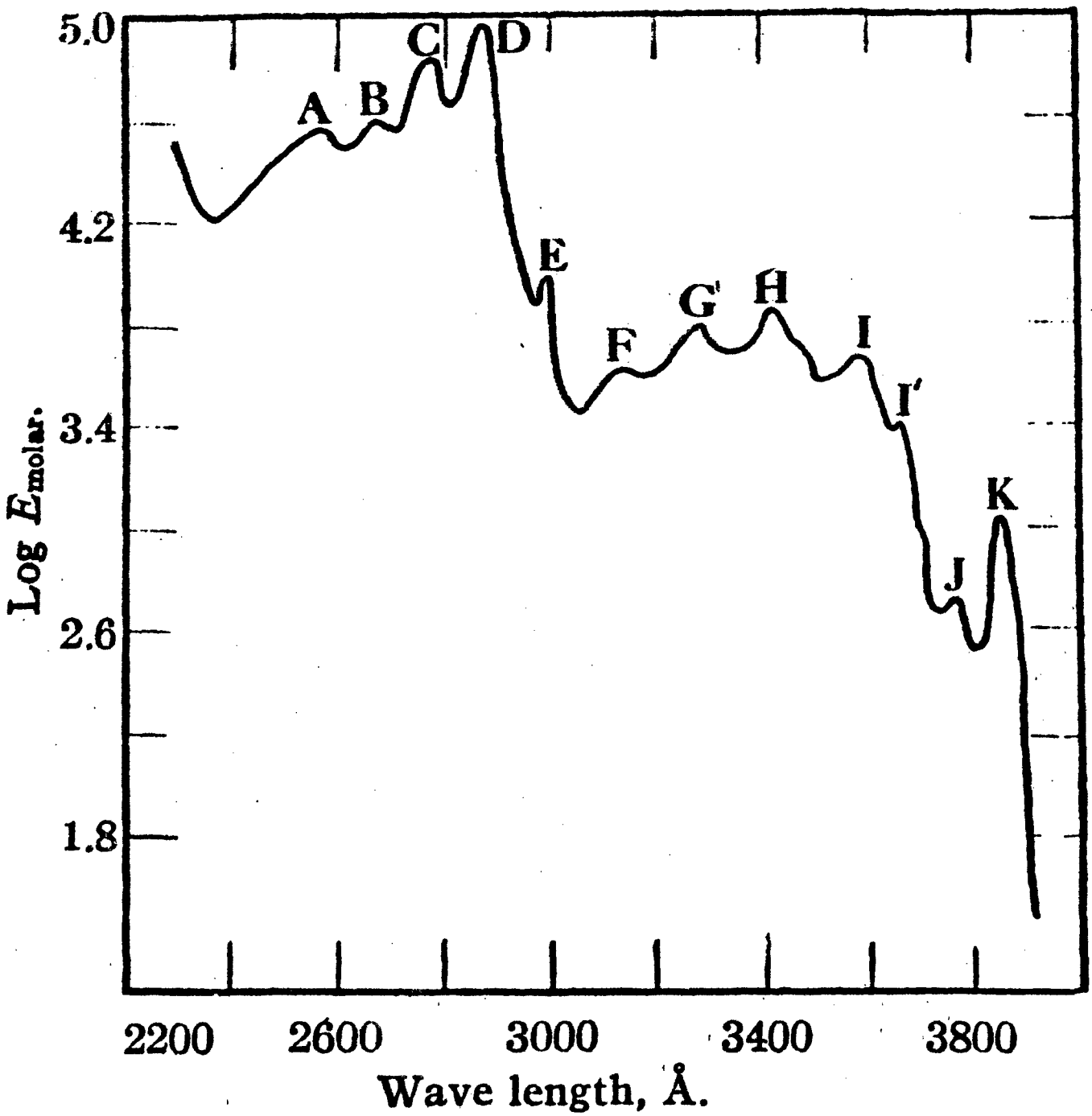
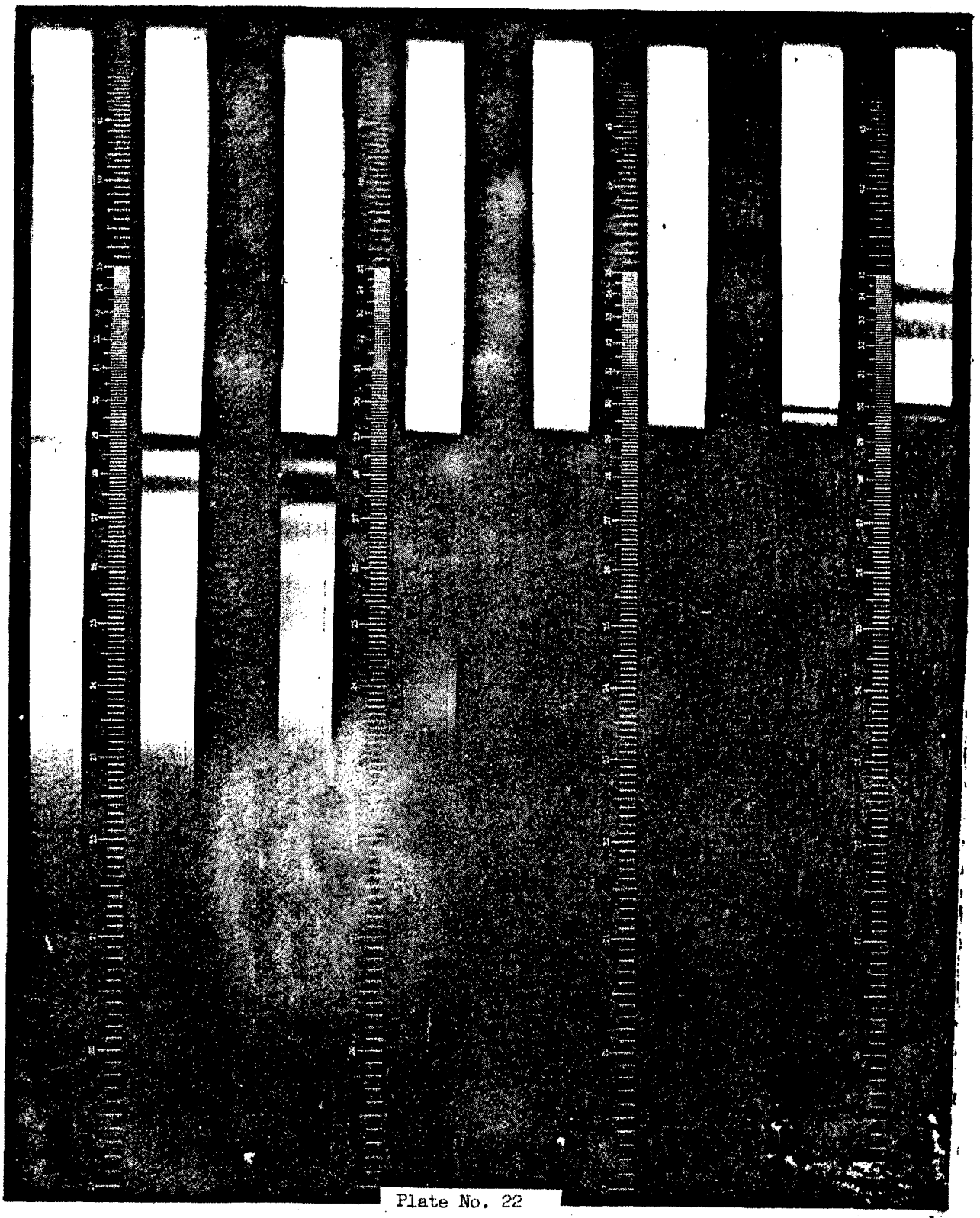


Fig. 1.—1,2-Benzanthracene.

Plate No. 21



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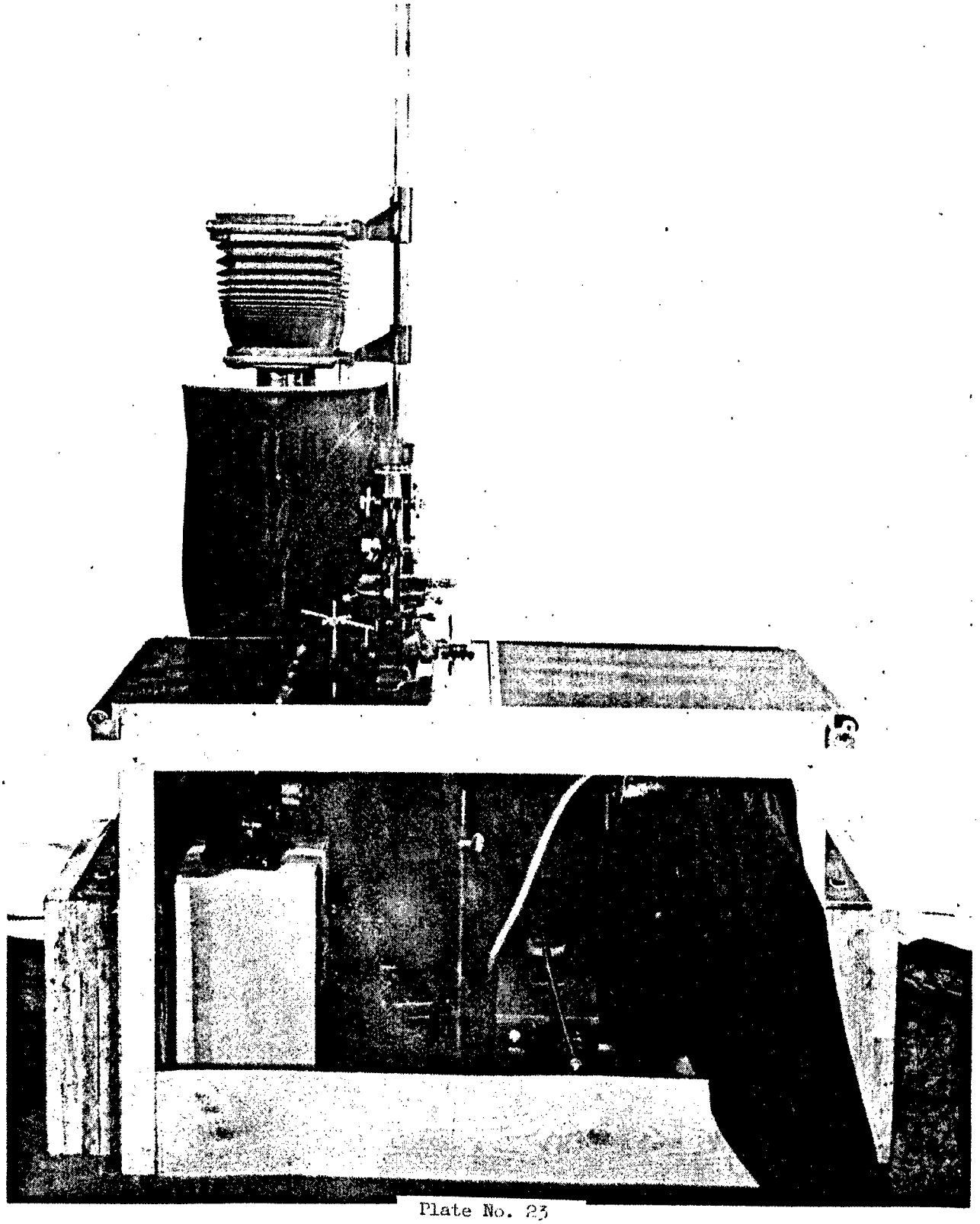

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